

CLAIMS

What is claimed is:

1. An attenuated bacterial mutant derived from a pathogenic bacterial strain, wherein said attenuated mutant has:

5 (i) a mutation of a gene selected from the group consisting of VIR1, VIR2, VIR3, VIR4, VIR5, VIR6, VIR7, VIR8, VIR9, VIR10, VIR11, VIR12, VIR13, VIR14, VIR15, VIR16, VIR17, VIR18, VIR19, VIR20, VIR21, VIR22, VIR23, VIR24, VIR25, VIR26, VIR27, VIR28, VIR29, VIR30, VIR31, VIR32, VIR33, VIR34, VIR35, VIR36, VIR37, VIR38, VIR39, VIR40, VIR41, VIR42, VIR43,
10 VIR44, VIR45, and VIR46; and

(ii) reduced inhibition of *Dictyostelium* amoeba growth when compared to the growth observed in the presence of an isogenic bacterial strain.

2. An attenuated bacterial mutant of claim 1, wherein said mutation is insertional
15 inactivation or a gene deletion.

3. An attenuated bacterial mutant of claim 1, wherein said mutant is a gram-negative bacteria.

20 4. An attenuated bacterial mutant of claim 3, wherein said attenuated gram-negative bacterial mutant is a *Pseudomonas* species.

5. An attenuated bacterial mutant of claim 4, wherein said *Pseudomonas* species is *Pseudomonas aeruginosa*.

25 6. An attenuated *Pseudomonas* mutant of claim 5, wherein said attenuated *Pseudomonas* mutant is selected from the group consisting of: MUT1; MUT2; MUT3; MUT4; MUT5; MUT6; MUT7; MUT8; MUT9; MUT10; MUT11; MUT12; MUT13; MUT14; MUT15; MUT16; MUT17; MUT18; and MUT19.

7. An attenuated bacterial mutant of claim 3, wherein said gram-negative bacterial mutant is a *Klebsiella* species.

5 8. An attenuated bacterial mutant of claim 7, wherein said *Klebsiella* species is *Klebsiella pneumoniae*.

9. An attenuated *Klebsiella* mutant of claim 8, wherein said attenuated *Klebsiella* mutant is selected from the group consisting of: MUT20; MUT21; MUT22; MUT23;
10 MUT24; MUT25; MUT26; MUT27; MUT28; MUT29; MUT30; MUT31; MUT32; MUT33; MUT34; MUT35; MUT36; MUT37; MUT38; MUT39; MUT40; MUT41; MUT42; MUT43; MUT44; MUT45; and MUT46.

10. A method for identifying an antimicrobial drug, said method comprising:

15 (a) contacting a candidate composition with at least one polypeptide encoded by a gene selected from the group consisting of VIR1, VIR2, VIR3, VIR4, VIR5, VIR6, VIR7, VIR8, VIR9, VIR10, VIR11, VIR12, VIR13, VIR14, VIR15, VIR16, VIR17, VIR18, VIR19, VIR20, VIR21, VIR22, VIR23, VIR24, VIR25, VIR26, VIR27, VIR28, VIR29, VIR30, VIR31, VIR32, VIR33, VIR34, VIR35,
20 VIR36, VIR37, VIR38, VIR39, VIR40, VIR41, VIR42, VIR43, VIR44, VIR45 and VIR46; and

(b) comparing the biological activity of said polypeptide in the presence and absence of said candidate composition, wherein alteration of the biological activity of said polypeptide indicates that said candidate composition is an
25 antimicrobial drug.

11. A method of claim 10, wherein said candidate composition contains at least two molecules.

12. A method of claim 10, wherein said candidate composition contains at least one molecule less than about 500 Daltons.

13. A method of claim 10, wherein said candidate composition contains at least
5 one molecule greater than about 500 Daltons.

14. A method of claim 10, wherein said candidate composition contains at least one molecule selected from a group consisting of a polypeptide, polysaccharide, lipid, nucleic acid, or combination thereof.

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15. A composition of claim 14, wherein said polypeptide is an immunoglobulin.

16. A method for identifying an antimicrobial drug, said method comprising:

(a) contacting a candidate composition with at least one polynucleotide
15 encoded by a gene selected from the group consisting of VIR1, VIR2, VIR3, VIR4,
VIR5, VIR6, VIR7, VIR8, VIR9, VIR10, VIR11, VIR12, VIR13, VIR14, VIR15,
VIR16, VIR17, VIR18, VIR19, VIR20, VIR21, VIR22, VIR23, VIR24, VIR25,
VIR26, VIR27, VIR28, VIR29, VIR30, VIR31, VIR32, VIR33, VIR34, VIR35,
VIR36, VIR37, VIR38, VIR39, VIR40, VIR41, VIR42, VIR43, VIR44, VIR45, and
20 VIR46; and

(b) comparing the expression of said polynucleotide in the presence and absence of said candidate composition, wherein alteration of the expression of said nucleotide indicates that said candidate composition is an antimicrobial drug.

17. A method of claim 16, wherein said candidate composition contains at least
25 two molecules.

18. A method of claim 16, wherein said candidate composition contains at least one molecule less than about 500 Daltons.

19. A method of claim 16, wherein said candidate composition contains at least one molecule greater than about 500 Daltons.

5 20. A method of claim 16, wherein said candidate composition contains at least one molecule selected from a group consisting of a polypeptide, polysaccharide, lipid, nucleic acid, or combination thereof.

21. A composition of claim 20, wherein said nucleic acid is a ribonucleic acid.

10 22. A nucleic acid of claim 21, wherein said nucleic acid is a small interfering ribonucleic acid.

23. A method for determining the degree of virulence of a pathogen in a subject,
15 said method comprising:

(a) measuring the level of expression of at least one polypeptide encoded by a gene selected from the group consisting of VIR1, VIR2, VIR3, VIR4, VIR5, VIR6, VIR7, VIR8, VIR9, VIR10, VIR11, VIR12, VIR13, VIR14, VIR15, VIR16, VIR17, VIR18, VIR19, VIR20, VIR21, VIR22, VIR23, VIR24, VIR25, VIR26,
20 VIR27, VIR28, VIR29, VIR30, VIR31, VIR32, VIR33, VIR34, VIR35, VIR36, VIR37, VIR38, VIR39, VIR40, VIR41, VIR42, VIR43, VIR44, VIR45, and VIR46, in a sample from the first subject; and

(b) comparing the amount of said polypeptide in said sample of step (a) to the amount of said polypeptide present in a control sample from a second subject
25 known not to have the presence of said pathogen, wherein an alteration in the expression level of said polypeptide in said first subject as compared to said control sample indicates the degree of virulence of said pathogen.

24. A method of claim 23, wherein said subject is a mammal.

25. A mammalian subject of claim 24, wherein said mammalian subject is a human.

5 26. A method for determining the degree of virulence of a pathogen in a subject, said method comprising:

(a) measuring the level of expression of at least one polynucleotide encoded by a gene selected from the group consisting of VIR1, VIR2, VIR3, VIR4, VIR5, VIR6, VIR7, VIR8, VIR9, VIR10, VIR11, VIR12, VIR13, VIR14, VIR15, VIR16, VIR17, VIR18, VIR19, VIR20, VIR21, VIR22, VIR23, VIR24, VIR25, VIR26, VIR27, VIR28, VIR29, VIR30, VIR31, VIR32, VIR33, VIR34, VIR35, VIR36, VIR37, VIR38, VIR39, VIR40, VIR41, VIR42, VIR44, VIR45, and VIR46, in a sample from the first subject; and

(b) comparing the amount of said polynucleotide in said sample of step (a) to the amount of said polynucleotide present in a control sample from a second subject known not to have the presence of said pathogen, wherein an alteration in the expression level of said polynucleotide in said first subject as compared to said control sample indicates the degree of virulence of said pathogen.

27. A method of claim 26, wherein said subject is a mammal.

28. A mammalian subject of claim 27, wherein said mammalian subject is a human.

29. An attenuated bacterial mutant of claim 1, wherein said mutant encodes and expresses a foreign antigen.

30. An attenuated bacterial mutant of claim 1, wherein said mutant contains a plasmid which encodes and expresses, in a eukaryotic cell, a foreign antigen.

31. A vaccine against a disease caused by a pathogenic microorganism comprising:

(a) a pharmaceutically effective dosage of one or more of the attenuated bacterial mutants of claim 1 and;

5 (b) a pharmaceutically acceptable diluent or carrier.

32. An attenuated bacterial mutant derived from a pathogenic bacterial strain, wherein said attenuated mutant has:

10 (i) a mutation of a gene selected from the group consisting of pchE, pchF, pchG, pchH, and pchI; and

(ii) reduced inhibition of *Dictyostelium* amoeba growth when compared to the growth observed in the presence of an isogenic bacterial strain.

33. A bacterial strain comprising an operon encoding a gene selected from the group consisting of VIR1, VIR2, VIR3, VIR4, VIR5, VIR6, VIR7, VIR8, VIR9, VIR10, VIR11, VIR12, VIR13, VIR14, VIR15, VIR16, VIR17, VIR18, VIR19, VIR20, VIR21, VIR22, VIR23, VIR24, VIR25, VIR26, VIR27, VIR28, VIR29, VIR30, VIR31, VIR32, VIR33, VIR34, VIR35, VIR36, VIR37, VIR38, VIR39, VIR40, VIR41, VIR42, VIR44, VIR45, and VIR46, wherein said bacterial strain includes a mutation that reduces expression of said gene relative to an isogenic bacterial strain lacking said mutation.

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34. A bacterial strain of claim 33, wherein said mutation reduces inhibition of *Dictyostelium* amoeba growth when compared to the growth of *Dictyostelium* amoeba in the presence of an isogenic bacterial strain lacking said mutation.